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CYSTINE OR DERIVATIVES OF CYSTINE

By FLORENCE ROY WHITE

A DISSERTATION SUBMITTED IN
PARTIAL FULFILMENT OF THE REQUIREMENTS FOR
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UNIVERSITY OF MICHIGAN

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THE METABOLISM OF SULFUR

XXIV. THE METABOLISM OF TAURINE, CYSTEIC ACID, CYSTINE, AND OF SOME PEPTIDES CONTAIN- ING THESE AMINO ACIDS

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The biological relation of taurine and the bile acids, of whose molecules this sulfonic acid is a component, to cystine is uncertain. The conversion of cystine to cysteic acid is readily accomplished in the laboratory, but the decarboxylation of this acid to form taurine, first demonstrated by Friedmann, has been difficult until recently, when White and Fishman (1) established the conditions necessary for this reaction. If taurine has its biological origin from cystine through cysteic acid, and if this is the normal path of catabolism of cystine, ready oxidation of the sulfur of taurine to sulfate, the end-product of cystine metabolism, should be observed. On the other hand, if taurine originates from a specialized path of catabolism of cystine, a reaction which results in a readily available supply of taurine for conjugation with cholic acid to form taurocholic and related acids, the complete oxidation of its sulfur to sulfate would not of necessity be anticipated.

Experimental data have shown that the sulfur of taurine and cysteic acid is not readily oxidized when these acids are administered to the common laboratory animals or to man. The experiments with cysteic acid are few and limited to studies with the dog. The literature concerned with the biological behavior of taurine has been summarized by one of us (2).

It has been shown that the oxidation of the sulfur of cystine does not proceed normally if the amino group is blocked in such a way as to inhibit deamination. In the sulfur-containing acids of the bile, the amino group of the taurine is in combination and does

not react with nitrous acid until liberated by acid hydrolysis. It seems possible that cystine may first conjugate with cholic acid and that the cystine, with its amino group thus blocked, may follow a metabolic path different from that of cystine itself, with the formation of a cysteic acid-cholic acid derivative and taurocholic acid. Taurine and cysteic acid thus in combination might be oxidized when the free acids might fail to be attacked in the animal organism. It is well established that pyrimidines in nucleoside combination, particularly cytosine and, to a lesser degree, uracil and thymine, are more readily and completely catabolized than the free pyrimidines (3). A similar picture might be presented in the oxidation of the biologically significant sulfonic acids, free and in combination.

The present studies are concerned with the distribution of urinary sulfur in the rabbit after the administration of peptides containing taurine or cysteic acid. In these compounds, the amino group is in combination as in the bile acids. It was felt that a study of these peptides might aid in the solution of the problem of the biological origin and significance of these two sulfonic acids.

A similar investigation of the urinary picture after the administration of a type of peptides little studied, the cystinyl peptides (4, 5), is also presented. Since glutathione readily decomposes to give glutamic acid or a derivative and cysteinylglycine, these peptides are of special interest. Moreover, the relation of cystine to the hypoglycemic activity of the polypeptide, insulin, lends additional interest to a study of the behavior of all peptides containing cystine.

EXPERIMENTAL

The general procedure and analytical methods were those commonly employed in this laboratory for studies of sulfur metabolism (6), male rabbits serving as the experimental animals. Cystine was prepared from hair; cysteic acid, by oxidation of cystine with bromine (7); taurine, by the method of Marvel and Bailey (8); glycylcysteic acid (9), glycyltaurine (9), and the cystinyl peptides (4), by the methods of White; and diglycylcystine, as described by Abderhalden and Spinner (10). All the compounds were of satisfactory purity, as evidenced by nitrogen and sulfur analyses.

Harris' titration (11) was also employed in the analyses of the peptides.

The compounds, either as such or as the sodium salt in a few cases, were dissolved in 10 to 15 cc. of water for oral administration and in 4 to 10 cc. for subcutaneous injection.

In order to make all the experiments as nearly comparable as possible, when the free sulfur-containing amino acids were to be administered, an amount of amino acid (glycine or alanine) equivalent to that present in the peptides was administered with the sulfur compound. Thus, the amounts of nitrogen and sulfur were the same in all experiments and the variable factor was the presence of the peptide linkage in the case of the peptides. The sulfur compounds were fed in amounts equivalent to 1.0 gm. of cystine (0.267 gm. of sulfur).

DISCUSSION

Cystic Acid, Taurine, and Peptides Containing These Acids (Table I)—In agreement with the older observations of Salkowski (12), the sulfur of orally administered taurine was oxidized in part to sulfate sulfur. However, since practically all the extra sulfur excreted after the subcutaneous injection of taurine was present in the organic sulfur fraction, it is believed that the oxidation observed after oral administration is to be ascribed to changes effected by the microflora of the intestine rather than to the action of cells of the organism proper. In feeding experiments, two variables, of unknown significance and not easily controllable, are introduced; *i.e.*, rate of absorption and activity of the intestinal microorganisms. These variables have not been considered adequately in many experiments, particularly in the studies of the metabolism of compounds of sulfur. There appears to exist some species difference, since Salkowski (12) and the present workers have observed a considerable degree of oxidation of the sulfur of orally administered taurine in the rabbit, while in the dog (13) and man the oxidation was so slight as to be practically within the limits of experimental error.

No evidence was obtained to indicate that the combination of taurine with glycine as a peptide¹ influenced the oxidation of its

¹ Ackermann (14) was able to isolate, from the urine of the dog, 8 per cent of the taurocyamine (guanyltaurine) fed (20 gm. in 4 days), but no studies of sulfur partition were made.

sulfur, varying degrees of oxidation being observed, as with free taurine, when the peptide was fed; but no oxidation was observed after subcutaneous injection.

It should be noted that in none of our studies here reported was any attempt made to determine whether deamination had occurred

TABLE I

Distribution of Extra Sulfur in Urine Excreted in 24 Hour Period Immediately Following Administration of Glycine and Taurine, Glycyltaurine, Glycine and Cysteic Acid, and Glycylcysteic Acid

Extra sulfur values are expressed as per cent of the total sulfur administered, the figures in parentheses showing the percentage distribution of the extra sulfur between sulfate and organic sulfur. The sulfur content of the compounds fed was, in all cases, 0.267 gm. The letters of the Experiment No. are used to designate the individual experimental animals, while the numeral refers to the number of the experiment. All experiments bearing the same letter were carried out with the same animal.

Experiment No.	Mode of administration	Compounds fed	Extra sulfur excreted		
			Total	Sulfate	Organic
M-1	Oral	Glycine, taurine	71.6	37.1 (52.0)	34.5 (48.0)
M-3	Subcutaneous	" "	68.3	2.0 (2.8)	66.3 (97.2)
M-18	"	" "	92.3	8.1 (8.8)	84.2 (91.2)
M-2	Oral	Glycyltaurine	46.5	29.7 (63.9)	16.8 (36.1)
M-5	"	"	59.3	35.4 (59.8)	23.9 (40.2)
O-16	"	"	52.2	17.6 (33.5)	34.6 (66.5)
M-4	Subcutaneous	"	80.1	-8.6(-10.8)	88.7(110.8)
M-19	"	"	98.5	6.2 (6.3)	92.3 (93.7)
L-8	Oral	Glycine, cysteic acid	77.8	21.4 (27.5)	56.4 (72.5)
N-10	Subcutaneous	" "	50.6	-6.8(-13.4)	57.4(113.4)
C-32	"	" "	53.6	2.1 (3.9)	51.5 (96.1)
L-9	Oral	Glycylcysteic acid	41.3	7.2 (26.4)	34.1 (73.6)
O-15	"	" "	45.8	12.9 (28.2)	32.9 (71.8)
N-11	Subcutaneous	" "	69.8	11.5 (16.6)	58.3 (83.4)
A-31	"	" "	70.7	25.3 (37.5)	45.4 (62.5)

or whether a carbamic acid derivative of taurine was excreted. The experiments are concerned solely with the fate of the sulfur-containing group of the molecule.

The results obtained with cysteic acid and its peptide are difficult of exact interpretation. After oral administration of both the

free cysteic acid and the peptide, a significant increase of oxidized (sulfate) sulfur was observed, amounting in three experiments recorded in Table I to slightly more than 25 per cent of the extra total sulfur excreted. This increased urinary excretion of oxidized sulfur was, however, distinctly less than that which was observed after oral administration of taurine or glycyltaurine. After the injection of the free cysteic acid, no increase in the excretion of extra sulfate sulfur was observed. When the peptide was injected, a mode of administration which excludes the intestinal factors previously discussed, some oxidation of sulfur was indicated. It is of interest to compare results of Experiments N-10 and N-11 in which the experimental animal was the same. It has been difficult to calculate exactly the extra sulfur excretion in certain of the glycylcysteic acid experiments, since some increase in the level of total nitrogen excretion was frequently observed in the injection experiments. This should result in an increased sulfur elimination not related to the utilization of the peptide. This objection applies to Experiment A-31. Nevertheless, we feel that there is some evidence of an oxidation of the cysteic acid in peptide linkage apart from changes produced by the intestinal microorganisms. Previous studies of the behavior of cysteic acid, as far as concerns the oxidation of its sulfur, have been limited to experiments with dogs (13).

The similarity of the changes in urinary sulfur distribution, after feeding the peptides and the sulfonic acids, suggested that enzymatic hydrolysis of the peptides might have occurred prior to absorption. The action of enzyme preparations from hog tissue, carboxypolypeptidase (pancreas), "erepsin," and aminopolypeptidase (intestine), and also glycerol extracts of liver and kidney on the two peptides was determined at a pH of 8.0 and at 30°. The activity of the preparations used was demonstrated by control experiments with the usual substrates. No hydrolysis by any of the enzymes of the intestinal tract studied was observed.² Glycyltaurine was split readily by the liver preparations, although not so readily as the leucylglycine. In experiments under com-

² The experiments with enzymes of the tissues of the hog were carried out by Dr. H. O. Calvery, with the methods of the Waldschmidt-Leitz group. We wish to express our indebtedness for conduct of these experiments and for advice in the other determinations.

parable conditions, 83 per cent cleavage of leucylglycine was observed as compared with a 29 per cent cleavage of glycyltaurine. The cleavages of the substrates by kidney extracts were 75 and 10 per cent, respectively. No significant hydrolysis of glycylcysteic

TABLE II

Distribution of Extra Sulfur in Urine Excreted in 24 Hour Period Immediately Following Administration of Cystine and Glycine, Cystine and Alanine, Cystinyldiglycine, Diglycylcystine, and Cystinyldialanine

Extra sulfur values are expressed as per cent of the total sulfur administered, the figures in parentheses showing the percentage distribution of the extra sulfur between sulfate and organic sulfur. The sulfur content of the compounds fed was, in all cases, 0.267 gm. The letters of the Experiment No. are used to designate the individual experimental animals, while the numeral refers to the number of the experiment. All experiments bearing the same letter were carried out with the same animal.

Experiment No.	Mode of administration	Compounds fed	Extra sulfur excreted		
			Total	Sulfate	Organic
L-14	Oral	Glycine, <i>l</i> -cystine	82.2	70.4 (86.0)	11.8 (14.0)
C-27	Subcutaneous	" "	59.4	52.6 (88.9)	6.8 (11.1)
C-23	Oral	<i>l</i> -Cystinyldiglycine	62.3	48.7 (78.5)	13.6 (21.5)
C-24	"	"	73.2	56.3 (77.2)	16.9 (22.8)
C-25	Subcutaneous	"	58.2	42.2 (72.8)	16.0 (27.2)
A-29	"	"	65.3	50.8 (78.0)	14.5 (22.0)
J-33	Oral	Diglycyl- <i>l</i> -cystine	64.3	51.8 (80.7)	12.5 (19.3)
M-6	"	"	61.1	47.4 (77.8)	13.7 (22.2)
O-17	"	"	33.7	31.9 (94.8)	1.8 (5.2)
J-34	Subcutaneous	"	56.0	36.1 (69.4)	19.9 (35.6)
M-7	"	"	56.7	25.5 (45.1)	31.2 (54.9)
N-12	"	"	65.3	37.6 (58.1)	27.7 (41.9)
A-20	Oral	<i>dl</i> -Alanine, <i>l</i> -cystine	70.8	45.2 (64.0)	25.6 (36.0)
A-22	"	" "	105.4	70.3 (66.8)	35.1 (33.2)
A-30	Subcutaneous	" "	53.6	45.9 (85.0)	7.7 (15.0)
A-21	Oral	<i>l</i> -Cystinyldi- <i>dl</i> -alanine	82.2	52.9 (64.8)	29.3 (35.2)
A-28	Subcutaneous	" "	70.2	51.9 (74.4)	18.3 (25.6)
C-26	"	" "	79.3	55.0 (73.2)	24.3 (26.8)

acid was obtained with any of the enzyme preparations. Similar results were obtained with preparations from rabbit tissue, although the hydrolysis of both leucylglycine and glycyltaurine was less extensive than with the preparations from hog tissue.

These experiments fail to indicate any opportunity for the hydrolysis of the sulfonic acid peptides in the gastrointestinal canal, except possibly by the action of microbial enzymes. In the tissues, ready hydrolysis of glycyltaurine would appear possible. Whether glycylcysteic acid can undergo cleavage is not apparent from our results.

Peptides Containing Cystine—In Table II we present data concerned with the distribution of the extra sulfur excreted in the urine after the oral and subcutaneous administration of *l*-cystine and *dl*-alanine or glycine, diglycyl-*l*-cystine and the cystinyl peptides, *l*-cystinyldiglycine and *l*-cystinyldi-*dl*-alanine. The recoveries of extra total sulfur and the percentage distribution of this extra sulfur are not significantly different, and demonstrate that the sulfur of both types of peptides is as readily oxidized as is the sulfur of cystine itself. There appears to be some indication of the excretion of a greater proportion of oxidized (sulfate) sulfur after the injection of *l*-cystinyldiglycine than after similar administration of diglycyl-*l*-cystine. However, since the experiments were not carried out with the same experimental animal, we do not regard this slight difference as particularly significant.

The data which indicate a ready utilization (as evidenced by oxidation of the sulfur) of peptides containing cystine are in harmony with the only similar study with which we are familiar, that of Abderhalden and Samuely (15). The sulfur of dialanyl-cystine and dileucylcystine was excreted as sulfate sulfur after oral or parenteral administration of these compounds to dogs. Evidence of utilization of cystine present in a peptide (diglycyl-*l*-cystine and di-*dl*-alanyl-*l*-cystine) has also been obtained in growth experiments with white rats by one of us (16). The cystine of the molecule of glutathione may also be utilized for purposes of growth (17). Oxidation of the sulfur of glutathione in the normal organism has also been studied,³ but the data are not very extensive (18).

In view of the usually accepted relationship of the sulfur-containing component of the insulin molecule to its hypoglycemic action, it is of interest to note that no indication of hypoglycemic

³ Studies with glycylcysteine (18) indicated a ready oxidation of peptides containing cysteine in the dog.

shock was observed in either injection or feeding experiments, despite the fact that each animal received the equivalent of 1.0 gm. of cystine in combination as a peptide. No determinations of blood sugar were made, however.

The hydrolysis of diglycyl-*l*-cystine by the enzymes of the gastrointestinal tract, previously demonstrated (19), was confirmed and cleavage of *l*-cystinyldiglycine by "erepsin" and aminopolypeptidase (intestine) and carboxypolypeptidase (pancreas) was established. It is evident that there is abundant opportunity for the hydrolysis of the cystine-containing peptides in the intestine.

SUMMARY

1. The oral administration of cysteic acid and taurine to rabbits led to a definite but variable increase in the excretion of oxidized (sulfate) sulfur of the urine as well as an increase in the organic sulfur fraction. This oxidation of the sulfur of these two biologically important sulfonic acids is believed to be related to the activity of the microflora of the intestine, since after parenteral (subcutaneous) introduction of these compounds, all the extra sulfur appeared in the organic sulfur fraction.

2. The excretion and distribution of the extra sulfur, after oral or parenteral administration of peptides containing glycine and cysteic acid or taurine, did not differ significantly from those observed after administration of the free sulfonic acids.

3. The sulfur of cystinyl peptides (*l*-cystinyldiglycine and *l*-cystinyldi-*dl*-alanine) was readily oxidized and excreted mainly as oxidized (sulfate) sulfur. No difference between the behavior of the cystinyl peptides studied and that of diglycylcystine in this respect was observed. No reactions resembling insulin shock were observed, although the peptides in amounts equivalent to 1.0 gm. of cystine were fed or injected.

4. *In vitro* studies indicated abundant opportunity for hydrolysis of both types of peptides containing glycine and cystine in the gastrointestinal canal.

5. Similar *in vitro* studies failed to demonstrate any hydrolysis of the peptides containing the sulfonic acids by the enzyme preparation of intestinal tract studied. Glycyltaurine was readily hy-

drolyzed by glycerol extracts of liver and kidney (hog and rabbit), but no cleavage of glycylcysteic acid was observed.

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